

25 years of thermomorphogenesis research - Milestones and perspectives

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Keywords: thermomorphogenesis, high temperature signalling, hypocotyl, phytohormones, thermosensor

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184 **Abstract**

185 In 1998, Bill Gray and colleagues showed that warm temperatures trigger *Arabidopsis*
186 hypocotyl elongation in an auxin-dependent manner. This laid the foundation for a vibrant
187 research discipline. With several active members of the ‘Thermomorphogenesis’ community,
188 we here reflect on 25 years of elevated ambient temperature research and look to the future.

189

190

191 **The beginning**

192 In the early days of molecular genetics, temperature-sensitive mutants became a tool for
193 dissecting molecular pathways. In the late 1970s, for example, essential genes regulating the
194 budding yeast secretory pathway were identified in this manner [1]. Being trained as a yeast
195 geneticist, Bill (William) Gray, a postdoc in Mark Estelle’s lab at Indiana University (USA), and
196 colleagues used a similar approach in *Arabidopsis thaliana* (hereafter *Arabidopsis*). Bill aimed
197 to identify temperature-sensitive alleles of *TRANSPORT INHIBITOR RESPONSE 1 (TIR1)*,
198 which was at the time an unknown auxin co-receptor. Although he was not successful at
199 identifying such *tir1* alleles, his observation that elevated temperature promotes auxin-
200 mediated hypocotyl elongation [2] would lay the foundation for a thriving research discipline
201 known now as thermomorphogenesis. This year the engaged and active
202 thermomorphogenesis community celebrates its 25th anniversary (Figure 1).

203

204 The Gray study provided the basis for a molecular framework to understand the physiological
205 responses to elevated ambient temperatures. Subsequently, work from the Franklin laboratory
206 provided important insights into the potential benefits that plants obtain from
207 thermomorphogenic growth patterns by showing that, in particular, petiole elongation and
208 hyponastic growth are associated with increased transpiration and lower leaf temperatures
209 (Figure 1A). This indicates that thermomorphogenesis stimulates leaf cooling by enhancing
210 evaporation, suggesting a possible functional relevance to plants, at least under laboratory
211 conditions [3]. However, in natural and agricultural settings the situation is undoubtedly more
212 complex as plants have to deal with multiple environmental factors at the same time (see also
213 below).

214

215 **Molecular signalling and thermosensing**

216 The publication of Gray’s seminal results [2] did not immediately spark follow-up studies.
217 Indeed, it was not until 2009, when the Franklin lab identified the bHLH transcription factor
218 PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) [4] as a key regulator of
219 thermomorphogenic signalling, paving the way for subsequent discoveries, including the
220 identification of the first thermosensors. In the same year, a second important study was
221 published, describing gibberellins and brassinosteroids as phytohormones that, in addition to
222 auxin, coordinate thermosensitive shoot growth [5], as we now know, downstream of PIF4.
223 These two papers [4,5], together with the finding that PIF4 directly regulates specific auxin
224 biosynthesis genes, like *YUCCA8* [6,7], inspired many scientists. More and more groups from
225 related disciplines (photobiology, phytohormone biology, natural variation, flowering
226 regulation, epigenetics, thermotolerance, cellular signalling, immunity, post-transcriptional
227 regulation, microRNA biogenesis) stepped in and started to elucidate how plants respond to
228 elevated ambient temperatures. Not least, the research interest was fueled by the emerging
229 awareness of rapid global warming and the need to harness crops to safeguard food security.
230 The prospect of contributing to climate change mitigation is still a major driver for many of the

231 authors of this paper to devote resources to gain fundamental knowledge of
232 thermomorphogenesis regulation and understanding its functional consequences.

233

234 Studying warm temperature signalling is not a trivial task because temperature, being
235 essentially molecular motion, is a versatile signal and has no ligand properties nor distinct
236 physical features. For many years, it therefore remained unclear whether or not specific
237 thermosensors had evolved in plants. Finding *bona fide* thermosensors has been, and still
238 remains a major goal. Although not entailing a dedicated sensor, a warm temperature relay
239 cascade was uncovered by the Wigge lab in 2010, showing that eviction of non-canonical
240 histone H2A.Z from chromatin of temperature-inducible genes is required for
241 thermomorphogenic responses [8]. Building on earlier work from the Whitelam, Schäfer and
242 Halliday labs, the Wigge and Casal labs showed that phytochrome B (phyB) is a thermosensor
243 using a combination of omics, biochemistry, spectroscopy and genetics, hence revealing that
244 thermomorphogenesis requires a surveillance system directly linked to light responses [9,10].
245 Subsequently, temperature-dependent phase transition of EARLY FLOWERING 3 (ELF3) into
246 biomolecular condensates and temperature-dependent conformational changes in *PIF7*
247 mRNA structure, resulting in enhanced translation, were shown to also sense temperature
248 changes [11,12]. Thus, various thermosensing mechanisms at the DNA, RNA and protein
249 levels have been uncovered and more are expected to be found.

250

251

252 **Where do we go from here?**

253 Based on the early findings on the involvement of PIF4, auxin and other hormones [2,4,5-7],
254 and by the identification of thermosensory mechanisms, thermomorphogenesis has become
255 an established field in the plant sciences. However, many important points remain to be
256 addressed. One major question is the spatial and temporal regulation of thermomorphogenic
257 responses across organs and tissues, down to potential cell type specificities. Although a
258 number of studies have specifically addressed this issue [e.g., 13], we are only now beginning
259 to understand communication of temperature signals within the plant. Another challenge is to
260 distinguish whether specific thermomorphogenic signalling events exist and to distinguish
261 these from thermodynamic effects on several (if not all) signalling networks, given that
262 temperature impacts every molecule and reaction in the plant, including enzyme activities.
263 This also raises the question of how cold, ambient warm and heat (tolerance) responses are,
264 if at all, connected. A gradient approach may reveal for example, whether cold regulators are
265 involved in warm temperature responses and *vice versa*, and thus if there exists a generic
266 response to temperature or whether distinct signalling branches deal with different
267 temperature cues. Part of the answer may be obtained by taking an epigenetic approach, as
268 regulation of different levels of histone H3 lysine 4 methylation appears to be a signalling hub
269 where diverse temperature cues converge [14].

270

271 At the organismal level, a potential pitfall of thermomorphogenesis research is that functional
272 hypotheses are relatively easy to formulate when only temperature is considered. For
273 example, temperature-induced root elongation may serve to reach deeper water to meet the
274 demands of increased transpiration and hyponasty is likely to reduce heat flux on the leaves.
275 However, the contribution of thermomorphogenesis and its component traits in natural
276 environments is complex and currently not well understood. One avenue of future research
277 should focus on understanding which thermomorphogenic responses actually contribute to
278 plant performance in the wild and/or agricultural environments, what fitness costs are

279 associated, and which regulators of the canonical or peripheral pathways are targeted by
280 natural selection to confer a selective advantage. While the community has so far gained
281 understanding of thermomorphogenesis under highly controlled conditions, it is time to expand
282 beyond the laboratory, to study thermomorphogenesis in wild species and in crops. It has been
283 reported that thermomorphogenesis does occur in several crop species such as cabbage,
284 tomato and wheat [15], yet the molecular mechanisms need to be further explored to be able
285 to contribute to the generation of climate-resilient varieties (Figure 1B). Emphasis should be
286 placed on the interaction with biotic signals and their potential trade-offs. Global warming will
287 increase pathogen pressure, highlighting the importance of better understanding the
288 interactions between temperature and the biotic environment, which may also apply *vice versa*
289 to microorganisms that positively influence plant performance in symbiotic interactions. In
290 addition, temperature will influence the way in which abiotic factors such as drought or salt
291 stress are perceived and dealt with. For example, while warm temperature episodes often co-
292 occur with drought, there is an apparent conflict in their optimal responses, since drought-
293 induced stomatal closure may prevent leaf cooling facilitated through increased
294 thermomorphogenesis-mediated transpiration. The cross-talk and potential priming role of
295 thermomorphogenesis on thermotolerance (e.g., do thermomorphogenesis signalling and
296 resulting phenotypes contribute to heat stress survival?) is another critical point that requires
297 further investigation.

298

299 Perhaps the greatest challenge for thermomorphogenesis research lies in translation: how do
300 we exploit conceptual breakthroughs in understanding temperature signalling to engineer
301 plant resilience in this era of unprecedented global warming? Despite excellent progress on
302 understanding the mechanisms underlying individual thermomorphogenic responses over the
303 last 25 years, we may have yet bigger discoveries to come.

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305

306 **Acknowledgements**

307 Many laboratories have made important contributions to the field. We sincerely apologize to
308 all those whose important work could not be included due to stringent space and referencing
309 constraints.

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347 **Figure legend**

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349 **Figure 1. Milestones in 25 years of thermomorphogenesis research** (A) Model
350 thermomorphogenic phenotypes represented on a stylised plant; arrows indicate thermo-
351 induced directions of the corresponding phenotypes. (B) Roadmap of selected important
352 research milestones, starting with the discovery that the auxin indole-3-acetic acid (IAA)
353 mediates temperature-dependent hypocotyl elongation and leading towards the generation of
354 future climate-resilient crops; colours of the centerline markings indicate the global
355 temperature change relative to the 1971-2000 average, based on information from
356 <https://showyourstripes.info/c/globe> with each stripe representing one year. Blue and red
357 colours indicate below-average and above-average temperatures, respectively, with darker
358 colours indicating greater deviations from the mean.

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